Journal of Pharmacokinetics and Biopharmaceutics, Vol. 9, No. 6, 1981

A Physiologically Based Pharmacokinetic Model for the Intraocular Distribution of Pilocarpine in Rabbits

Susan C. Miller,[1,3] Kenneth J. Himmelstein,[1,2] and Thomas F. Patton[1,4]

Received February 10, 1981—Final July 28, 1981

This report presents a mathematical model which has been developed to describe the intraocular disposition of pilocarpine following topical dosing in rabbits. The model uses experimentally determined parameters such as rates of tissue uptake of drug and equilibrium distribution coefficients. Differential mass balance equations for pilocarpine in the cornea, aqueous humor, iris-ciliary body, and lens were written and solved numerically. Measured tear concentrations, following topical dosing with pilocarpine, were fit by a monoexponential curve and used as the forcing function for the model. By using a combination of known physiological and experimentally determined parameters, predictions of intraocular tissue levels of pilocarpine were made. These predictions were then compared to experimentally determined concentration-time profiles.

KEY WORDS: pilocarpine; intraocular tissues; pharmacokinetic model; mass transfer; distribution coefficient.

INTRODUCTION

Several pharmacokinetic models have been developed in recent years to describe the time course of pilocarpine in the eye following topical dosing (1–3). For the most part, the predictive ability of these models has been limited to descriptions of aqueous humor levels of pilocarpine obtained after topical ocular dosing. For pilocarpine, and probably a number of other ocular drugs, evidence suggests that drug levels achieved in tissues surrounding the anterior chamber are markedly different from aqueous

[1]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66045.
[2]Department of Chemical and Petroleum Engineering, The University of Kansas, Lawrence, Kansas 66045.
[3]Present address: University of Minnesota, College of Pharmacy, Minneapolis, Minnesota 55455.
[4]Author to whom inquiries should be addressed.

0090-466X/81/1200-0653$03.00/0

humor drug levels (2). Since pharmacologic sites of action are often located in these tissues, it becomes particularly important to understand the distribution and movement of drugs in these areas.

In 1978, a preliminary physiologically based pharmacokinetic model was developed to describe the ocular disposition of pilocarpine in albino rabbits (1). In the model, the eye was depicted as consisting of two basic compartments, the tears or precorneal fluid, and the aqueous humor. This model was the first attempt to describe drug disposition in the eye by something other than a one-compartment open model. Although simplified in form, the model's major advantage over a classical pharmacokinetic description was that it used physiologically meaningful and experimentally verifiable parameters to describe ocular drug disposition.

More recently, Lee and Robinson used a similar approach to provide a more extensive description of the ocular pharmacokinetics of pilocarpine (3). In addition to predicting drug concentration-time profiles in the aqueous humor, their model provided a description of drug movement through the cornea, as well as a more complete representation of the factors influencing precorneal drug loss.

To date, however, no attempt has been made to comprehensively describe the disposition of pilocarpine in the internal tissues of the eye. The model presented herein was developed to provide a better understanding of the fate of pilocarpine, once it reaches the aqueous humor. To that end, a combination of *in vitro* and *in vivo* techniques were used to quantitate the various phenomena affecting the movement and distribution of pilocarpine within the eye.

EXPERIMENTAL

Concentration Versus Time Profiles

All rabbits used in the study were male, New Zealand white (Small Stock Industries, Pea Ridge, Ark.), ranging in age from 56 to 65 days. Prior to experimentation, the rabbits were housed in standard cages and allowed food and water *ad libitum*. During the experiments, rabbits were kept in restraining boxes which maintained their normal upright position.

Radiolabeled ^{3}H(G)-pilocarpine alkaloid (New England Nuclear, Boston, Mass.) (10.0 Ci/mmol) was received in ethanol and was evaporated to dryness several times prior to use to remove any solvent that had become tritiated by exchange (4). Using TLC (5), the radiochemical purity was determined to be 97%. Radiolabeled pilocarpine nitrate solutions were prepared in isotonic Sorensen's phosphate buffer. The final *p*H of the drug solutions was 7.2.

Rabbits were dosed topically with 25 μl of 1.00×10^{-2} M pilocarpine nitrate. At various times postinstillation, rabbits were sacrificed with an intravenous overdose of pentobarbital sodium. Experimental details for the determination of pilocarpine in ocular fluids and tissues have been reported elsewhere (6).

Precorneal Loss of Pilocarpine

Lacrimal fluid samples were collected into 1 μl microcapillary tubes after the topical instillation of 25 μl of 1.00×10^{-2} M pilocarpine nitrate, *p*H 7.2. Samples were taken at 1 min intervals for the first 5 min postinstillation.

The filled capillary tubes were placed directly in polyethylene minivials containing 5 ml of Aquasol® (New England Nuclear, Boston, Mass). Prior to counting, samples were stored in the dark for 24 hr. After correcting for background radiation, drug concentrations in the samples were determined using standard drug solutions, prepared in the same manner.

*p*H Determination in Lacrimal Fluid

The *p*H of the normal, resident lacrimal fluid was determined *in vivo* by the use of sensitive *p*H indicator sticks (ColorpHast, E. Merck, Darmstadt, Germany), cut into strips approximately 1×5 mm. In a second series of experiments, the change in lacrimal fluid *p*H, as a function of time, was determined following the topical instillation of 25 μl of 1.00×10^{-2} M pilocarpine nitrate, *p*H 7.2.

In Vitro Incubation of Ocular Tissues with Aqueous Pilocarpine Solutions

The following experiments were designed to estimate the rate of exchange (clearance) of pilocarpine between an aqueous solution and ocular tissues such as the cornea, iris-ciliary body, and lens. Due to the existence of parallel pathways *in vivo*, it is often difficult to quantitate the kinetics of drug movement within the eye for a specific pathway. Therefore, it was felt that an isolated, *in vitro* system might be useful for estimating the rate of drug transfer between ocular fluids such as the tears or aqueous humor and a particular ocular tissue.

A second aspect of these experiments was to determine the extent of interaction of pilocarpine with these tissues by quantitating their equilibrium distribution coefficients. If the drug-tissue interaction is linear, that is, the fraction of drug bound remains relatively constant over a wide range of drug concentration, the product of the equilibrium distribution coefficient

and the total volume of a particular tissue, summed over all of the tissues, can provide an estimate of the volume of distribution for drug in the eye. A mathematical description of the experimental system and the method used to obtain the parameters of interest are given in Appendix A.

Immediately after sacrifice of the animal, the cornea, iris-ciliary body,[5] and lens were dissected from each eye, blotted, and transferred to tared, V-shaped glass vials. The vials were reweighed, and the wet tissue weights were determined by difference. Two hundred and fifty μl of 5.00×10^{-5} M pilocarpine nitrate (pH 7.2) were added, and the vials were sealed with teflon-lined screw caps. The samples were incubated at 33°C for varying lengths of time up to a period of 5 hr. At a preselected time, the drug solution (incubate) was drawn off the tissue and transferred to a glass vial. A 150 μl aliquot of this solution was placed in a microultrafiltration cell (MRA, Clearwater, Fla.), which was preassembled with a 10,000 MW cutoff filter (PM-10, Amicon Corporation, Lexington, Mass.). The cell was pressurized with nitrogen, and the filtrate from each sample was collected. The samples were filtered to remove any water soluble protein that might have diffused from the tissues during incubation. The presence of these proteins in the incubate could cause an overestimate of free drug concentration since these soluble proteins have the potential to bind drug. Filtrate aliquots were transferred to minivials containing 5 ml of Aquasol®. Prior to counting, samples were stored in the dark for 24 hr. CPM were corrected for background radiation, and drug concentrations in the incubates were determined using standard drug solutions treated in the same manner. Tissue drug concentrations were determined by difference.

In Vitro Metabolism of Pilocarpine by Rabbit Ocular Tissues

The possibility of metabolism or degradation occurring during the course of these experiments was investigated by determining the radiochemical purity of the pilocarpine nitrate solutions before and after incubation with the cornea, iris, and lens. This was accomplished using TLC and radiochromatographic scanning of the developed plates. The extent of metabolism/degradation was assessed by comparing the radiochemical purity of the drug solution before and after incubation for the period of time required for attainment of apparent equilibrium for each of the tissues.

For the cornea, it appeared that about 90% of the radioactivity in the incubate was still in the form of pilocarpine after 2 hr of incubation at 33°C. After incubation of an iris for a period of 3 hr, approximately 70% of the radioactivity in the incubate was in the form of pilocarpine.

[5]In the following discussion, any reference to observations made for the iris implies that the tissue was comprised of both the iris and ciliary body.

Metabolism appeared to be the slowest in the lens, with 90% of the radioactivity still attributable to pilocarpine after an incubation period of 4 hr.

From these studies, it was apparent that some metabolism did occur during the course of the *in vitro* incubation studies. For the cornea and lens, the metabolism did not appear to be extensive during the time course of these experiments. For the iris, approximately 30% of the drug appeared to be metabolized within 3 hr. Thus the value of the equilibrium distribution coefficient for pilocarpine between aqueous solutions and the iris could be in error if the apparent distribution characteristics of pilocarpine and its metabolites differ significantly.

RESULTS

Concentration versus time profiles for pilocarpine in the aqueous humor, cornea, iris-ciliary body, and lens, following the topical instillation of 25 μl of 1.00×10^{-2} M pilocarpine nitrate, *p*H 7.2, have been reported elswhere (6). Table I lists the average concentration of pilocarpine in the precorneal area as a function of time under the same dosing conditions. The change in concentration of pilocarpine in the precorneal area, as a function of time, appeared to follow first-order behavior. The apparent first-order rate constant associated with the loss of pilocarpine from the precorneal area was 0.42 min^{-1}, in good agreement with the value of 0.43 min^{-1} reported by Lee and Robinson (3).

Table I. Average Concentration[a] of Pilocarpine in the Precorneal Area Following the Topical Instillation of 25 μl of 1.00×10^{-2} M Pilocarpine Nitrate, *p*H 7.2

Time (min)	Pilocarpine, in μg per ml of tears
1	880 (160)[b]
2	500 (150)
3	420 (110)
4	220 (100)
5	160 (50)

[a] Concentrations are based on pilocarpine alkaloid and refer to the average of seven determinations.
[b] Numbers in parentheses refer to the standard error of the mean.

Table II. Lacrimal Fluid *p*H as a Function of Time Following the Topical Instillation of 25 μl of 1.00×10^{-2} M Pilocarpine Nitrate, *p*H 7.2

Time (min)	Lacrimal fluid *p*H[a]
1	7.18 (0.016)[b]
3	7.18 (0.045)
6	7.16 (0.018)
10	7.25 (0.027)
15	7.35 (0.042)

[a] Lacrimal fluid *p*H is reported as the average of eight determinations.
[b] Numbers in parentheses refer to the standard error of the mean.

The lacrimal fluid *p*H, observed as a function of time, following the administration of 25 μl of 1.00×10^{-2} M pilocarpine nitrate, *p*H 7.2, is reported in Table II. Normal lacrimal fluid *p*H in 60 day old rabbits was determined to be 7.49 (SEM = 0.03, $n = 16$). This value is in reasonable agreement with the value of 7.42 recently reported by Keller *et al.* (7). From the data in Table II, it can be seen that upon the administration of pilocarpine nitrate, the *p*H of the lacrimal fluid was depressed to that of the instilled solution. Even as long as 15 min postinstillation, the *p*H of the tear fluid had not returned to its normal value.

Figure 1 is a plot of C_T/C_I versus time for the incubation of corneas in aqueous solutions of pilocarpine, where C_T is the total concentration of drug in the tissue, and C_I is the total concentration of drug in the incubate at any given time. This plot can be described by the following theoretical equation (taken from Appendix A):

$$\frac{C_T}{C_I} = \frac{V_I R(1 - e^{-xt})}{V_I + V_T R\, e^{-xt}} \tag{1}$$

where

$$x = \frac{\gamma(V_I + V_T R)}{V_I V_T R}$$

In Fig. 1, the open circles represent the average experimental values for C_T/C_I, based on one to four determinations at each time point. Bars represent the standard error of the mean. The solid line represents the values for C_T/C_I generated by solving Eq. (1) as a function of time, using

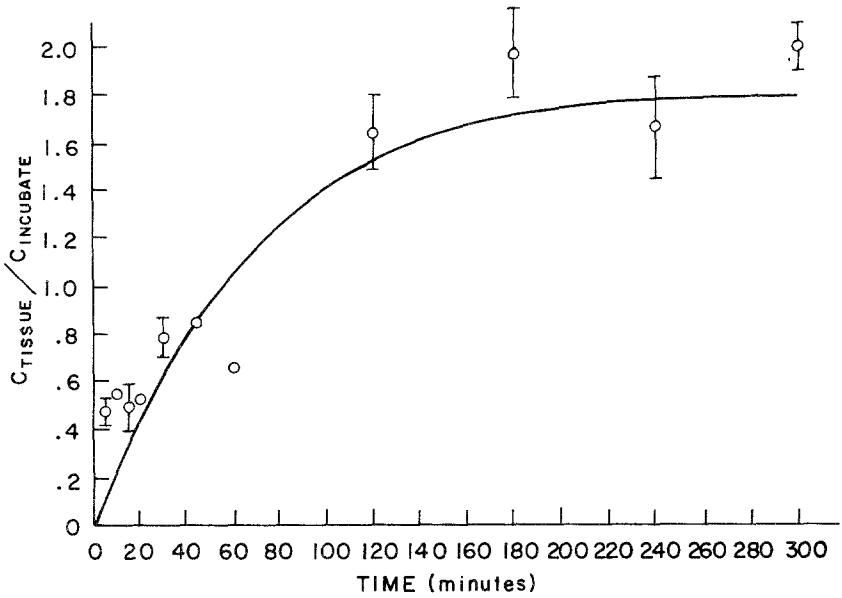

Fig. 1. Concentration (cornea)/concentration (incubate) versus time for the *in vitro* equilibration of ocular tissue from 60 day old rabbits (open circles represent experimental data, bars indicate standard error of the mean, solid line represents the theoretical curve).

the experimentally determined values of R and γ, which were obtained as described below.

For the cornea, a t-test was used to determine that there was no statistically significant difference between C_T/C_I at 120 min and C_T/C_I at 300 min (the highest and lowest values). Therefore, for the cornea, all of the individual values of C_T/C_I obtained at 120, 180, 240, and 300 min were averaged to obtain an estimate of R. The calculated equilibrium distribution coefficient for the cornea was 1.81.

The values of C_T/C_I obtained for the iris are shown in Fig. 2. The iris tissues took slightly longer than the corneas to reach an apparent equilibrium value, since the quotient of C_T/C_I did not approach a constant until about 3 hr. The equilibrium distribution coefficient determined for the iris, based on all of the individual observations at times 180 min and beyond, was 2.00.

A plot of C_T/C_I versus time for the lens is shown in Fig. 3. From this plot, it can be seen that the lens required a longer time than either the cornea or iris to attain apparent equilibrium. The equilibrium distribution

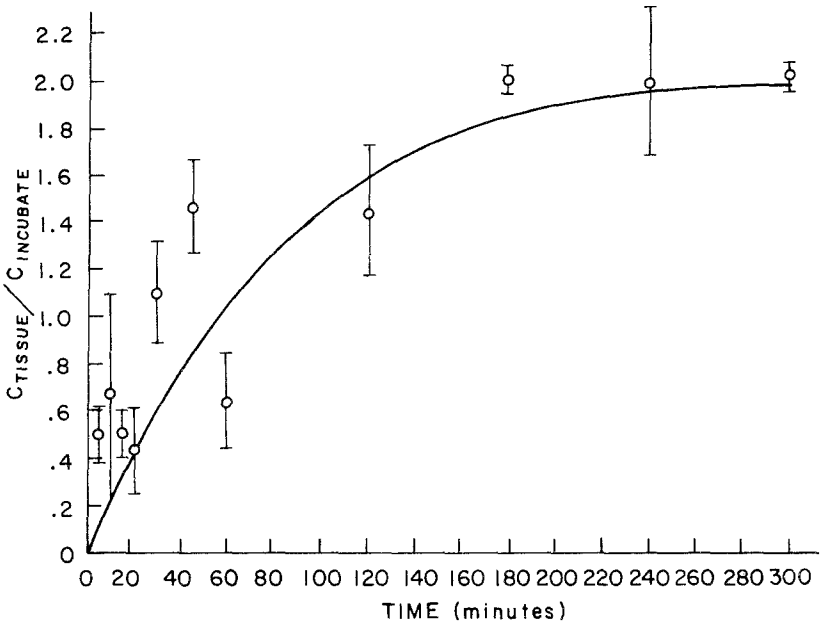

Fig. 2. Concentration (iris-ciliary body)/concentration (incubate) versus time for the *in vitro* equilibration of ocular tissue from 60 day old rabbits (open circles represent experimental data, bars indicate standard error of the mean, solid line represents the theoretical curve).

coefficient for the lens was determined on the basis of C_T/C_I values observed at 240 and 300 min. The calculated value was 1.00.

From these studies, it appeared that mong these tissues, pilocarpine has the greatest affinity for the iris-ciliary body, followed by the cornea and the lens. Due to the ionizability of pilocarpine, its partitioning behavior between aqueous solutions and ocular tissues might be *p*H dependent. This aspect, however, was not investigated. Since the purpose of these studies was to estimate the partitioning behavior with respect to the aqueous humor and internal ocular tissues, all tissues were incubated with a solution buffered at a *p*H of 7.2.

The interaction of pilocarpine with plasma protein has been investigated, and over the 100-fold range of concentration (1×10^{-6} to 1×10^{-4} M), the fraction bound was found to be approximately 0.27 (8). Due to this finding, for the purposes of this study, it was assumed that the interaction of pilocarpine with ocular tissues is also linear. Linear drug-tissue interaction, however, is an assumption that should be verified, and the binding of drugs to ocular tissues is an area of research that warrants further

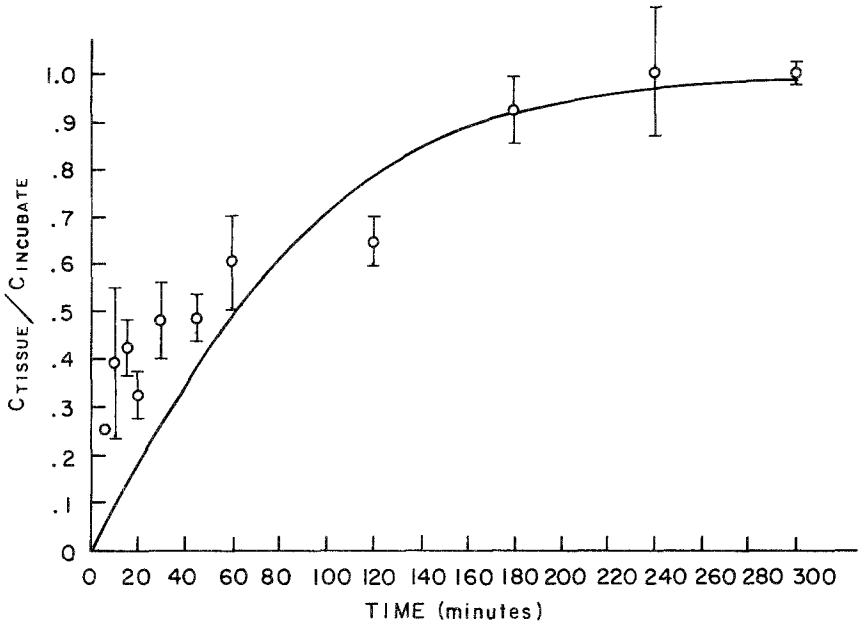

Fig. 3. Concentration (lens)/concentration (incubate) versus time for the *in vitro* equilibration of ocular tissues from 60 day old rabbits (open circles represent experimental data, bars indicate standard error of the mean, solid line represents the theoretical curve).

investigation. The results of the equilibrium distribution coefficient determinations for pilocarpine between aqueous solutions buffered at *p*H 7.2 and the cornea, iris-ciliary body, and lens are summarized in Table III.

The product of these equilibrium distribution coefficients and the total tissue volume was used to estimate an apparent tissue volume of distribution for pilocarpine. These individual volumes were summed, over all of the tissues, to obtain an apparent ocular volume of distribution for pilocarpine. The apparent volume of distribution for pilocarpine calculated in this manner was about 1.8 ml This value was obtained by multiplying the equilibrium distribution coefficients determined for the cornea, iris-ciliary body, and lens by the average weight of these tissues (6). Volumes of distribution were determined for the aqueous humor and the vitreous humor using the average volumes of these fluids (6, 9), assuming a distribution coefficient of one.[6]

[6]To assume a distribution coefficient of 1 is probably reasonable, since these tissues are >99% water.

Table III. Equilibrium Distribution Coefficients for Pilocarpine Between Ocular Tissues and *p*H 7.2 Phosphate Buffer

	Equilibrium distribution coefficient (R)	95% Confidence interval for R	Time required to reach apparent equilibrium
Cornea	1.81 (0.10)[a] [16][b]	1.61↔2.01	2 hr
Iris-ciliary body	2.00 (0.08) [12]	1.82↔2.18	3 hr
Lens	1.00 (0.05) [7]	0.87↔1.13	4 hr

[a] Numbers in parentheses refer to the standard error of the mean.
[b] Numbers in brackets refer to the number of determinations on which R was based.

In order to estimate γ, the mass transfer coefficient for drug between the incubate and a particular ocular tissue, the value of R, and the equilibrium distribution coefficient must be known. The following equation (from Appendix A) describes the concentration of drug in the incubate as a function of time:

$$C_I = \frac{C_0 V_I}{V_I + V_T R} + \frac{C_0 V_T R}{V_I + V_T R} \exp\left[-\left(\frac{\gamma(V_I + V_T R)}{V_I V_T R}\right) t\right] \tag{2}$$

Equation (2) was rearranged, as shown below, to obtain a form suitable for plotting:

$$\frac{R V_I V_T}{V_I + V_T R} \ln\left[\frac{C_I(V_I + V_T R) - C_0 V_I}{C_0 V_T R}\right] = -\gamma t \tag{3}$$

If the left-hand side [LHS] of this equation is plotted against time, the negative of the slope of the line is equal to γ.

Since R is an experimentally obtained parameter, an error is associated with its determination. Table III lists the 95% confidence interval for the experimentally determined values of R. To obtain estimates for the value of γ, the best estimate of R and its upper and lower 95% confidence limits were used in Eq. (3). In all cases, V_I, the volume of the incubate, was 0.250 ml, and C_0, the initial drug concentration in the incubate, was 5.00×10^{-5} M. C_I, the total concentration of drug in the incubate at any given time t, and V_T, the volume of the tissue, were assigned various values based on the individual, observed concentrations and experimentally determined weights.[7]

[7] It was assumed that the densities of the drug solutions were equal to 1 g/ml.

For each observation, the three values of R for a particular tissue were used in Eq. (3), and the LHS was regressed on time using restricted least-squares regression analysis (10) such that the line passed through the origin. For each tissue, these analyses were done using each of the individual observations for C_I, for all times up to and including the apparent equilibration time (see Table III).

For each tissue, use of the calculated R value and its upper and lower 95% confidence limits provided three different estimates for γ. In addition, due to scatter in the experimental data, each of the three regression coefficients for a particular tissue had an error term associated with its determination. This error term was used to calculate the 95% confidence interval for each of the three regression coefficients. These calculations lead to an upper and lower 95% confidence limit for each of the original three regression coefficients, obtained by using the three estimates of R (i.e., the average and upper and lower 95% confidence limits) in Eq. (3) for each of the ocular tissues.

As a result of the above analysis, for each tissue, nine regression coefficients were generated. Three of these arose from assigning three different values to R in Eq. (3). The other six coefficients resulted from the 95% confidence limits (upper and lower) of the three regression coefficients originally determined. Table IV is a summary of the extreme upper and lower 95% confidence limits (see below) for γ as well as the best estimate for this parameter [obtained by using the average values for R listed in Table III in Eq. (3)].

Table IV. Mass Transfer Coefficients, γ, Associated with the Overall Movement of Pilocarpine from Aqueous solutions (pH 7.2) into Ocular Tissues[a]

	Lower limit	Estimate	Upper limit
Cornea	0.75	1.06	1.24
	(2.01)[b]	(1.81)	(1.81)
	[21][c]	[21]	[21]
Iris-ciliary body	0.47	0.92	1.24
	(2.18)	(2.00)	(2.00)
	[30]	[27]	[27]
Lens	1.36	2.08	2.34
	(1.13)	(1.00)	(0.87)
	[31]	[30]	[27]

[a] Parameters are reported in units of μl/min.
[b] Numbers in parentheses refer to the value of R used in the regression analysis.
[c] Numbers in brackets refer to the number of observations used in the calculation of the regression line.

If the value of R used in the LHS of Eq. (3) was not sufficiently large, the numerator of the bracketed term became negative, which made it meaningless since the natural logarithm of this term had to be taken. In all instances when this occurred, that data point was not used in the analysis. For all of the tissues, the extreme lower limit of γ arose from the lower 95% confidence limit of the regression coefficient obtained by using the upper 95% confidence limit for R in Eq. (3). It would be expected that the extreme upper limit for γ would be obtained from the upper 95% confidence limit of the regression coefficient obtained by using the lower 95% confidence limit for R in Eq. (3). However, for the cornea and lens, the extreme upper limit for γ arose from the use of the average value for R in Eq. (3). For these tissues, some of the data were excluded from the analysis as described above. For each of the ocular tissues, the individual observations for C_I were used in Eq. (3), along with the average value of R, to evaluate the LHS of this expression as a function of time to obtain the best estimate for γ.

The best estimate of γ listed in Table IV for the individual ocular tissues and the equilibrium distribution coefficient listed in Table III were used in Eq. (1) to generate the theoretical values of C_T/C_I shown previously by solid lines in Figs. 1–3. Before use in the model, the mass transfer coefficients obtained from the *in vitro* studies were adjusted for surface area and pH of the bathing solution as discussed below.

MODEL

Development

A schematic of the proposed model for the intraocular disposition of pilocarpine is shown in Fig. 4. The eye was depicted as consisting of five major compartments; the precorneal area, cornea, aqueous humor, iris-ciliary body, and lens.

In the model, it was assumed that drug movements between compartments were reversible processes. As discussed below, drug elimination from the eye (leading ultimately to the systemic circulation) was assumed to occur only from the aqueous humor and iris-ciliary body. In Fig. 4, aqueous humor turnover is indicated by the parameter Q_{AH}, and k_{AH} represents facilitated drainage due to the pharmacologic action of pilocarpine on outflow. Drug loss from the iris-ciliary body, attributed to intraocular venous circulation, is represented by K_I.

Although admittedly a simplified approach, all compartments in the model were treated as being homogeneous. For each of the compartments,

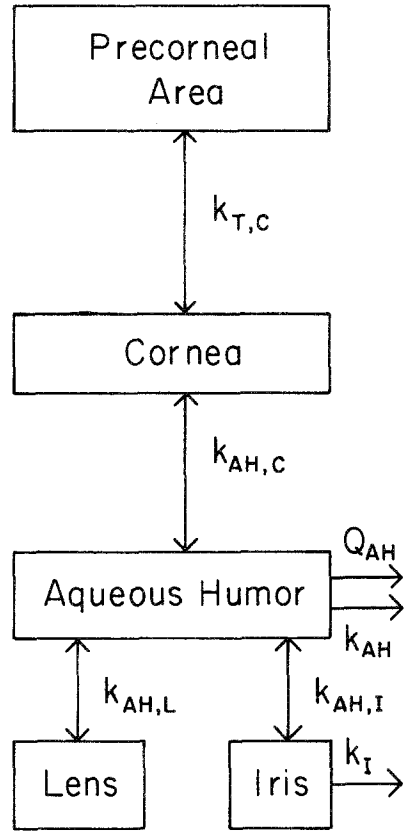

Fig. 4. Schematic of the proposed model for the intraocular disposition of pilocarpine.

differential mass balance equations were written for pilocarpine as described in Appendix B.

Total Ocular Clearance of Pilocarpine

In all previous models for the ocular disposition of pilocarpine (1–3), drug elimination from the eye was assumed to occur from either the precorneal area or anterior chamber. In the model proposed by Himmelstein *et al.* (1), a value of 0.66 μl min^{-1} was assigned to tear turnover (11). Drug loss from the anterior chamber was described by a lumped, first-order clearance parameter and assigned a value of 7.5 μl min^{-1}. This average clearance was obtained by multiplying the volume of the anterior chamber

by the apparent elimination rate constant for pilocarpine from the aqueous humor, using experimentally observed aqueous humor concentrations followed for a period of 2 hr after topical dosing. It now appears, however, that the apparent elimination phase of pilocarpine from the aqueous humor during short-term studies (i.e., up to 2 hr postinstillation) reflects both distribution and elimination (2,12). It has been shown (2) that if aqueous humor drug levels observed following topical dosing are followed for longer periods of time (e.g., up to 12 hr), the declining portion of the concentration-time curve is multiexponential. A similar observation has been made when aqueous humor levels of pilocarpine are followed for long periods of time following intracameral injection.[8] For both routes of drug administration, the terminal decline in aqueous humor levels of pilocarpine appeared to have an associated first order rate constant which is about 0.004 min^{-1}. The aqueous humor concentrations of pilocarpine with which this rate constant were associated were of the order of magnitude of 10^{-8} M.

In the model proposed by Lee and Robinson (3), drug loss from the precorneal area was described as occurring by two processes: normal tear turnover and nonproductive elimination. Tear turnover was assigned a value of 0.66 $\mu l\ min^{-1}$ (11), and a value of approximately 8 $\mu l\ min^{-1}$ was assigned to nonproductive drug loss. The elimination of pilocarpine from the aqueous humor was described as occurring at a rate of about 4 $\mu l\ min^{-1}$. Therefore, it initially appeared that the total clearance of pilocarpine from the eye as described in the model by Lee and Robinson (3) was about 12–13 $\mu l\ min^{-1}$. However, their studies also indicated that the clearance of pilocarpine between the precorneal area and the cornea was about 0.1 $\mu l\ min^{-1}$. Thus if the postdistributive elimination of pilocarpine from the aqueous humor is considered, the movement of drug from the cornea to the precornial area, or "back-diffusion," would represent a rate-limiting step for drug elimination from the eye. Therefore, despite the fact that the precorneal clearance of pilocarpine appeared to be very large, at late times, clearance from the precorneal area contributed little to the total ocular clearance of pilocarpine. Therefore, precorneal drug elimination was not explicitly included in the present development.

If it was assumed that by 12 hr the ocular distribution of pilocarpine was complete, and that aqueous humor concentrations of pilocarpine on the order of 10^{-8} M were too low to exert a pharmacologic effect on aqueous humor turnover (see below), it appears that the total ocular clearance of pilocarpine was approximately 8 $\mu l\ min^{-1}$. This clearance value was obtained by multiplying the apparent ocular volume of distribution for pilocarpine by its terminal elimination rate constant. Normal aqueous

[8] R. D. Gokhale and T. F. Patton, unpublished data.

humor turnover can only account for approximately 3 μl min^{-1} of this total clearance (13). Other possible pathways of drug elimination include precorneal loss, vitreous humor turnover, and drug removal through intraocular venous circulation. The recent work of Lee and Robinson (3) indicated that the precorneal clearance of pilocarine via nonproductive pathways is quite rapid. Nonetheless, these pathways cannot contribute substantially to the terminal elimination of drug from the aqueous humor since the clearance of pilocarpine between the cornea and tear fluid is a rate-limiting process.

In rabbits, it has been shown that some loss of ocular fluid does occur via posterior drainage of the vitreous humor (14). However, little quantitative information is available regarding the movement of drugs into and from this fluid. In addition, despite the relatively large volume of the vitreous humor, under the dosing condition considered here, vitreous humor drug concentrations are low, relative to concentrations in the other ocular tissues (6). On the average, drug present in the vitreous humor accounts for less than 5% of the total amount of drug in the eye (6). Therefore, it was assumed that vitreous humor turnover was insignificant when considering the total ocular clearance of pilocarpine.

It has been shown that blood flows through the rabbit uvea at approximately 2 ml min^{-1} (15). However, since the blood-aqueous barrier is located in this tract, it is unlikely that drug clearance through the uvea would approach this rate. Nonetheless, it is likely that some drug is removed from the eye by this route. In the model, it was assumed that in addition to aqueous humor turnover, drug loss via intraocular blood flow represented the only major route of drug elimination from the eye. Its contribution to total drug clearance was assessed to be approximately 5 μl min^{-1}. This represents the difference between the total ocular clearance of pilocarpine (at aqueous humor drug levels of 10^{-8} M or less) and clearance due to normal aqueous humor turnover.

Influence of Pilocarpine on Aqueous Humor Outflow

Comparison of a number of aqueous humor concentration-time profiles for pilocarpine, obtained under a variety of topical dosing conditions in rabbits (i.e., different drug concentrations and instilled solution volumes), suggests that the ocular pharmacokinetics of pilocarpine are dose dependent (1,16).[9] For short duration studies, the rate constants describing the post-peak apparent elimination vary as a function of aqueous humor drug levels. That is, the higher the level of pilocarpine in the aqueous humor, the more

[9]T. F. Patton, unpublished data.

rapidly the drug is eliminated. It follows, therefore, that for a given dosing volume, the AUC would not be directly proportional to the applied dose. This has also been observed.

It is now recognized that what was termed the elimination phase in many previous studies is actually a postpeak *apparent* elimination phase that reflects both distribution and elimination (12). Nonetheless, assuming that tissue distribution processes are first-order, the overall dose-dependent elimination of pilocarpine (i.e., the higher the drug concentration, the more rapid the elimination) is consistent with the fact that pilocarpine can enhance aqueous humor outflow.

To quantitate the pharmacologic effect of pilocarpine on aqueous humor turnover, it was necessary to identify dose/response parameters. It was not appropriate to use the topically applied dose since the fraction of dose absorbed is dependent on the instilled solution volume (16). Thus the apparent peak concentration was used as a measure of the effective dose. The pharmacologic site of action of pilocarpine is presumed to be in the iris-ciliary body. Nonetheless, it can be assumed that an apparent equilibrium exists between drug levels in this tissue and the aqueous humor. Little information exists relative to the pharmacokinetics of pilocarpine in the iris. Thus it had to be assumed that drug levels in the iris could be correlated with aqueous humor drug levels.

The pharmacologic "response" of the eye to doses of pilocarpine was determined as follows: "postpeak" clearances were obtained by multiplying experimentally observed apparent drug elimination rate constants (1,16),[10] reflective of both tissue distribution and drug elimination, by the volume of distribution. From these values, the value of 8 μl min^{-1} (the clearance of pilocarpine in the absence of a pharmacologic effect) was subtracted to obtain the residual clearance. The residual clearance was used as a measure of "response," and was attributed to the ability of pilocarpine to facilitate aqueous humor outflow. The residual clearance was plotted against the logarithm of the apparent peak concentration for a given profile as shown in Fig. 5. Although the data available are somewhat limited, there does appear to be a linear relationship between the residual clearance and the logarithm of the apparent peak concentration observed after a variety of dosing conditions. From this relationship, it appears that the residual clearance or pharmacologic effect of pilocarpine on aqueous outflow would cease to exist at aqueous humor concentrations of pilocarpine less than 0.015 μg ml^{-1}. This is consistent with the previous assumption that aqueous humor concentrations of pilocarpine that are of the order of 10^{-8} M do not have a significant effect on aqueous humor turnover.

[10]See footnote 5 above.

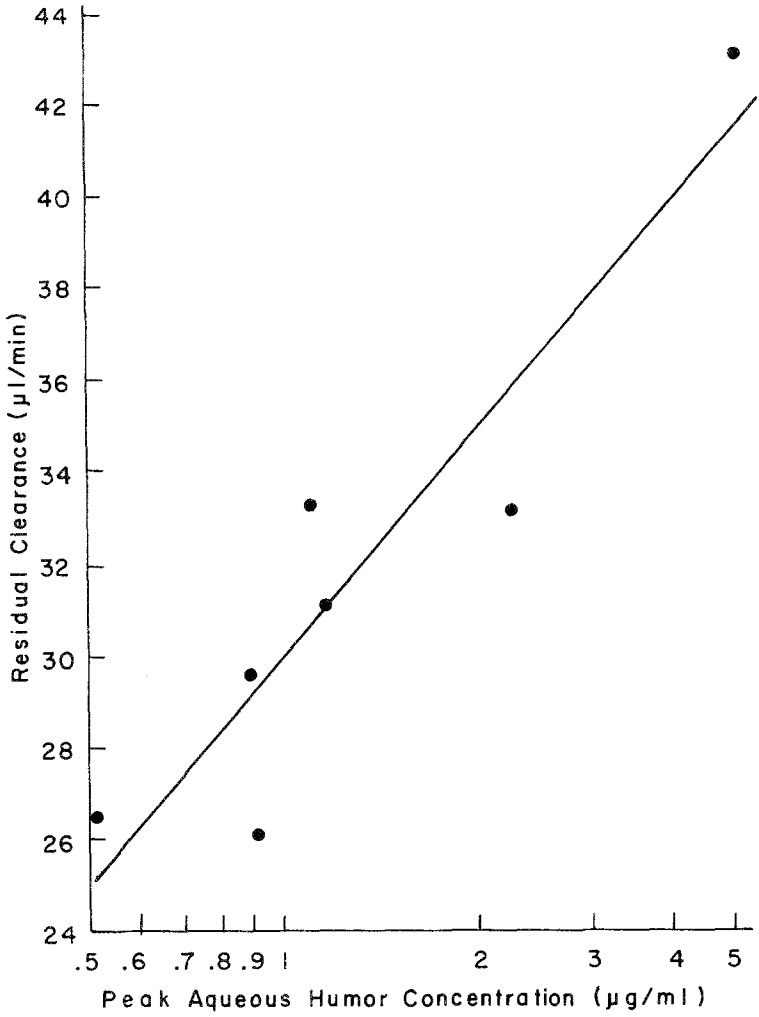

Fig. 5. Residual ocular clearance of pilocarpine versus the logarithm of the peak aqueous humor concentration (residual clearance = $16.5 \log C_{AH} + 30$, $p < 0.005$).

Model Evaluation

Table V lists the values assigned to the various parameters in the solution of the model. Values for tissue weights (volumes) were taken from ref. 6. These parameters were used in the equation set given in Appendix

Table V. Model Parameters Used to Describe the Intraocular Disposition of Pilocarpine

Parameter	Value[a]
$K_{T,C}$	0.545
$K_{AH,C}$	11.0
Q_{AH}	3.11
K_{AH}[b]	1.7×10^{-4}; $[16.5 \log C_{AH} + 30]$
$K_{AH,L}$	0.990
$K_{AH,I}$	1.80
K_I	5.95

[a] Units of all parameters are $\mu l\ min^{-1}$.
[b] If C_{AH} was less than 0.015 $\mu g\ ml^{-1}$, K_{AH} was assumed to be zero.

B (Table A1). The equation set was solved, simultaneously, using Hamming's predictor-corrector method (17) and a digital computer (Honeywell Model 66/60). Figure 6 is a comparison of the model-predicted and experimentally determined concentration-time profiles. Except for $K_{AH,C}$, which is the mass transfer coefficient associated with the movement of pilocarpine between the cornea and aqueous humor, all of the mass transfer coefficients used were within the 95% confidence limits of the estimates obtained for these parameters from the *in vitro* equilibrium studies.

From Fig. 6, it can be seen that reasonable agreement was obtained between the experimentally observed concentrations of pilocarpine in the cornea and aqueous humor and those predicted by the model. In this figure, drug concentrations refer to pilocarpine alkaloid. For the iris, the model predicted profile appeared to be slightly lower than that observed experimentally. This discrepancy may have been due to the fact that either the intrinsic mass transfer coefficient or equilibrium distribution coefficient or both of these parameters for pilocarpine and the iris, determined from the *in vitro* studies, may be in error, possibly because of drug metabolism in this tissue. For the lens, experimental drug concentrations at early times were reasonably predicted; however, at late times the model over-predicted the experimentally observed drug concentrations. This is probably due, at least in part, to the fact that the lens can also exchange drug with the vitreous humor, which was not included in the model. Additionally, while the lens was considered as a homogeneous compartment, its size and structure might demand a distributed parameter approach. Due to the size and structure of the lens, it may be possible that during the *in vitro* equilibration studies, only a pseudoequilibrium was reached. That is, pilocarpine may have equilibrated with the external tissues of the lens

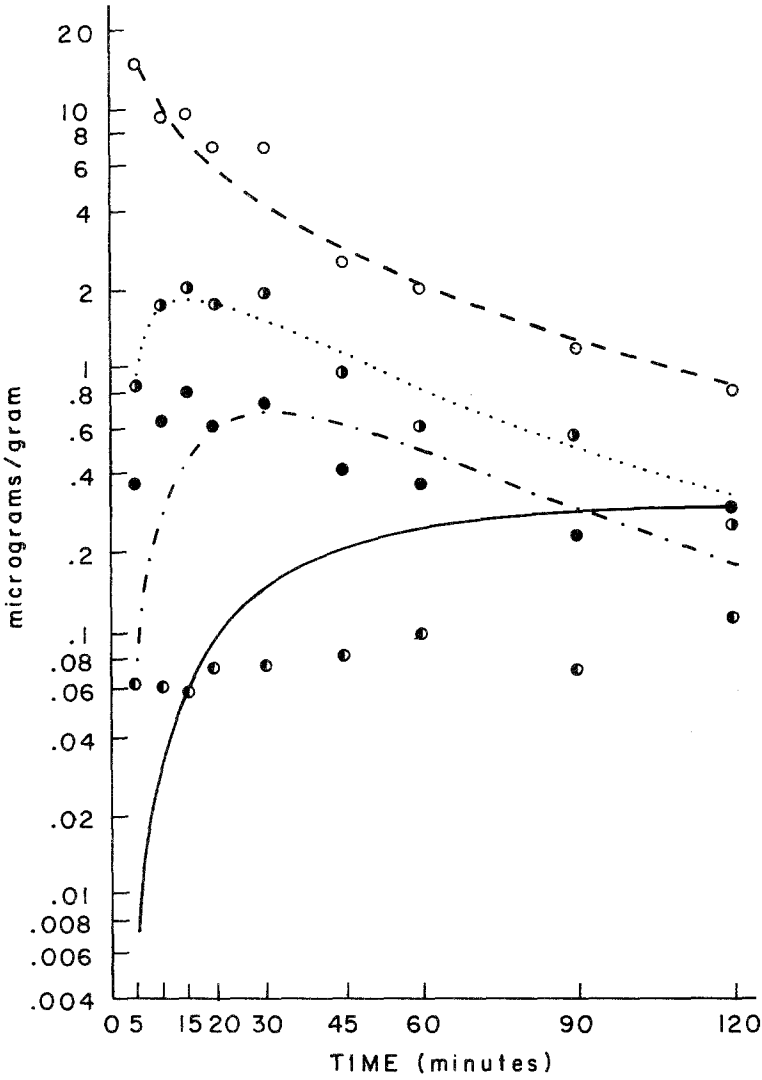

Fig. 6. comparison of model-predicted and experimentally determined concentrations of pilocarpine in ocular tissues following the topical instillation of 25 μl of 1.00×10^{-2} M pilocarpine nitrate, *p*H 7.2. Cornea: open circles, experimental; dashed line, model predicted. Aqueous humor: right-filled circles, experimental; dotted line, model predicted. Iris-ciliary body: solid circles, experimental; dash-dot line, model predicted. Lens: left-filled circles, experimental; solid line, model predicted.

without reaching a uniform distribution in the entire tissue. Similarly, it is possible that the amount of drug present in this tissue following topical instillation, during the time course of this study (2 hr), was also not uniformly distributed.

CONCLUSIONS

It has been shown that the intraocular disposition of pilocarpine in rabbits can be described by a physiologically based pharmacokinetic model. *In vitro* tissue incubation studies appear to provide reasonable estimates of the rate of drug uptake by individual ocular tissues as well as estimates of the equilibrium distribution coefficients for drug between an aqueous solution and ocular tissues. The model confirms experimental findings that pilocarpine is widely distributed in intraocular tissues and predicts, with some accuracy, the time course of drug in these tissues. Since pharmacologic sites of action are often located in these tissues, such predictive capability is particularly significant. Further, it appears that aqueous humor turnover accounts for less than half of the total intraocular clearance of pilocarpine, the remainder possibly due to removal via local circulation. The model does not account for drug metabolism in ocular tissues, nor is the vitreous humor included as a physiologic compartment. In addition, an explicit representation of the precorneal area is not included. These refinements as well as separate descriptions of the movement of nonionized and monoprotonated pilocarpine should allow prediction of intraocular tissue concentrations of drug under a variety of dosing conditions.

APPENDIX A

Mathematical Description of the *In Vitro* System Used for Determining the Equilibrium Distribution Coefficient and Mass Transfer Coefficient for Pilocarpine Between Aqueous Solutions and Ocular Tissues

Figure A1 is a schematic of the experimental system used to quantitate the rate of uptake of pilocarpine by ocular tissues such as the cornea, iris-ciliary body, and lens. The system is composed of two volumes, the drug solution (incubate) and one of the individual ocular tissues. C_I and C_T represent the total concentration of drug in the incubate and tissue, respectively, at any given time t. V_I and V_T are the respective volumes of the two compartments.

In the model, it was assumed that the rate of change of mass in each of the compartments was first-order with respect to pilocarpine and that the compartments were homogeneous. Equations (A1) and (A2) can be

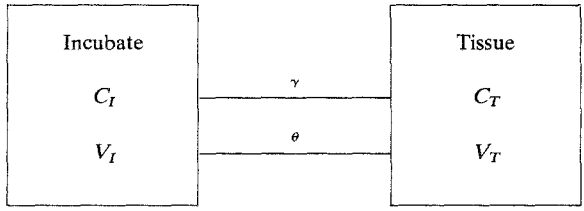

Fig. A1.

used to describe the rate of change of mass of pilocarpine in the incubate and tissue compartments, respectively:

$$\frac{dC_I V_I}{dt} = -\gamma C_I + \theta C_T \tag{A1}$$

$$\frac{dC_T V_T}{dt} = -\theta C_T + \gamma C_I \tag{A2}$$

In these equations, γ and θ are hybrid mass transfer coefficients which reflect the total clearance of pilocarpine between the two compartments.

At equilibrium,

$$\frac{dC_I}{dt} = \frac{dC_T}{dt} = 0 \tag{A3}$$

Therefore, either Eq. (A1) or (A2) can be rearranged to define the equilibrium distribution coefficient R for the total concentration of drug in the incubate and tissue:

$$\left[\frac{C_T}{C_I}\right]_{EQ} = \frac{\gamma}{\theta} = R \tag{A4}$$

This equilibrium distribution coefficient can then be used to redefine θ, in terms of γ and R, thereby simplifying Eqs. (A1) and (A2). The simplified equations can be readily solved, using Laplace transforms and the boundary conditions of $C_T = 0$ and $C_I = C_0$ at $t = 0$. This results in the following explicit expressions for the total concentration of drug in the incubate and tissue as a function of time:

$$C_I = \frac{C_0 V_I}{V_I + V_T R} + \frac{C_0 V_T R}{V_I + V_T R} \exp\left[-\left(\frac{\gamma(V_I + V_T R)}{V_I V_T R)}\right) t\right] \tag{A5}$$

$$C_T = \frac{C_0 V_I R}{V_I + V_T R}\left\{1 - \exp\left[-\left(\frac{\gamma(V_I + V_T R)}{V_I V_T R}\right) t\right]\right\} \tag{A6}$$

Dividing Eq. (A6) by (A5) yields the following expression:

$$\frac{C_T}{C_I} = \frac{V_I R(1-e^{-xt})}{V_I + V_T R\, e^{-xt}} \tag{A7}$$

where

$$x = \frac{\gamma(V_I + V_T R)}{V_I V_T R}$$

which describes the distribution of pilocarpine between the incubate and tissue as a function of time. If C_T/C_I is plotted versus time, the plateau portion of the curve can be extrapolated to the y axis to determine $(C_T/C_I)_{EQ}$, the equilibrium distribution coefficient. Once the value for R has been obtained, the value of γ can be determined using the following rearranged form of Eq. (A5):

$$\frac{V_I V_T R}{V_I + V_T R} \ln\left[\frac{C_I(V_I + V_T R) - C_0 V_I}{C_0 V_T R}\right] = -\gamma t \tag{A8}$$

Since γ was defined as the mass transfer coefficient for the total movement of pilocarpine from the incubate into the tissue, it is, in fact, a hybrid parameter. It can be expressed in terms of its components as shown below:

$$\gamma = \gamma_B f_B + \gamma_{BH^+} f_{BH^+} \tag{A9}$$

where γ_B and γ_{BH^+} are the intrinsic mass transfer coefficients associated with the movement of nonionized and monoprotonated pilocarpine, and f_B and f_{BH^+} represent the fraction of the total drug present in each of these forms. At pH 7.2, $f_B \cong 0.7$ and $f_{BH^+} = 0.3$. No information is available, however, relative to the magnitudes of γ_B and γ_{BH^+}. Due to the lipoidal nature of biological membranes, it is probably reasonable to assume that γ_B is greater than γ_{BH^+}. Also, f_B is approximately two times greater than f_{BH^+}. Therefore, it was assumed that the overall rate of change of pilocarpine between the incubate and tissue was due primarily to the movement of nonionized pilocarpine, or that

$$\gamma \cong \frac{\gamma_B K_a}{K_a + [H^+]} \tag{A10}$$

Keeping this assumption in mind, the mass transfer coefficient γ_B associated with the movement of nonionized pilocarpine can be approximated by multiplying the negative of the slope of the line obtained from Eq. (8) by a factor of $(K_a + [H^+])/K_a$, using $\mathrm{p}K_a = 6.85$ (18).

The mass transfer coefficients determined for pilocarpine, between buffered aqueous solutions and ocular tissues, are dependent on the surface area of the tissue that is exposed to the bathing solution. Therefore, in an attempt to approximate *in vivo* conditions more closely, the values reported in Table IV were corrected for surface area of exposure before use in the model. For the cornea and lens, the mass transfer coefficients were halved since only one side of these tissues is exposed to aqueous humor. *In vivo*, however, nearly all surfaces of the iris are bathed by aqueous humor; therefore, it was felt that the mass transfer coefficient determined *in vitro* would provide a reasonable estimate of the *in vivo* parameter.

APPENDIX B

Differential Mass Balance Equations for Pilocarpine in the Cornea, Aqueous Humor, Iris-Ciliary Body, and Lens Describing the Intraocular Disposition of Pilocarpine

The differential mass balance equations for pilocarpine in the individual ocular tissues, depicted in the model shown in Fig. 4, are given in Table A1. The driving force for drug movement was established by mathematically describing the experimentally observed tear concentration-time profile C_T

Table A1. Differential Mass Balance Equations for Pilocarpine in Ocular Tissues

Cornea

$$\frac{dC_c}{dt} = \frac{K_{T,C}\left[\frac{K_a}{K_a+[H^+]_T}\right]\left[C_T - \frac{C_c}{R_c}\right] + K_{AH,C}\left[\frac{K_a}{K_a+[H^+]_{AH}}\right]\left[C_{AH} - \frac{C_c}{R_c}\right]}{V_c}$$

Aqueous humor

$$\frac{dC_{AH}}{dt} = \frac{\frac{K_a}{K_a+[H^+]_{AH}}\left[K_{AH,C}\left(\frac{C_c}{R_c} - C_{AH}\right) + K_{AH,I}\left(\frac{C_I}{R_I} - C_{AH}\right) + K_{AH,L}\left(\frac{C_L}{R_L} - C_{AH}\right)\right] - C_{AH}[Q_{AH} + K_{AH}]}{V_{AH}}$$

Iris-ciliary body

$$\frac{dC_I}{dt} = \frac{\frac{K_a}{K_a+[H^+]_{AH}}\left[K_{aH,I}\left(C_{AH} - \frac{C_I}{R_I}\right) - K_I\frac{C_I}{R_I}\right]}{V_I}$$

Lens

$$\frac{dC_L}{dt} = \frac{K_{aH,L}\left[\frac{K_a}{K_a+[H^+]_{AH}}\right]\left[C_{AH} - \frac{C_L}{R_L}\right]}{V_L}$$

for pilocarpine following the topical instillation of 25 μl of 1.00×10^{-2} M pilocarpine nitrate. Solution of the model indicated that only tear drug concentrations during the first 5 min postinstillation were critical in establishing the concentration gradient of pilocarpine between the precorneal area and the cornea.

In the model, it was assumed that transport of ionized pilocarpine was negligible compared to the movement of nonionized drug. Therefore, to describe transport processes, the total concentration of pilocarpine was converted to the concentration of nonionized pilocarpine. Values for tear *p*H were determined experimentally, and the *p*H of the aqueous humor was set at 7.57 (13). Ocular tissue *p*H data were not available; therefore, it was assumed that drug transfer at tissue surfaces (i.e., between compartments) was controlled by the *p*H of the bathing fluid (e.g., tears or aqueous humor).

If more information was available, relative to the pH of the individual ocular tissues, as well as the relative magnitudes of the intrinsic mass transfer coefficients associated with the movement of nonionized and ionized drug, C_I and C_T could be described as a function of time using Eqs. (A11) and (A12):

$$\frac{dC_I}{dt} = \frac{1}{V_I}\left[\gamma_B\left(\frac{f_B^T C_T}{R} - f_B^I C_I\right) + \gamma_{BH^+}\left(\frac{f_{BH}^T + C_T}{R} - f_{BH^+}^I C_I\right)\right] \quad \text{(A11)}$$

$$\frac{dC_T}{dt} = \frac{1}{V_T}\left[\gamma_B\left(f_B^I C_I - \frac{f_B^T C_T}{R}\right) + \gamma_{BH^+}\left(f_{BH^+}^I C_I - \frac{f_{BH^+}^T C_T}{R}\right)\right] \quad \text{(A12)}$$

In these equations, f_B and f_{BH^+} again represent the fraction of total drug present in the neutral and monoprotonated forms, respectively. The superscripts on these terms indicate whether the fraction is determined as a function of tissue *p*H or *p*H of the bathing fluid. Experiments to determine the magnitude of these parameters are currently in progress.

ACKNOWLEDGMENTS

This work was supported by a grant from The University of Kansas General Research Fund, grant EY01945 from NIH-National Eye Institute (TFP), and was also aided by the American Foundation for Pharmaceutical Education, William E. Weiss Memorial Fellowship, 1978–1980 (SCM).

REFERENCES

1. K. J. Himmelstein, I. Guvenir, and T. F. Patton. Preliminary pharmacokinetic model of pilocarpine uptake and distribution in the eye. *J. Pharm. Sci.* **67**:603–606 (1978).
2. M. C. Makoid and J. R. Robinson. Pharmacokinetics of topically applied pilocarpine in the rabbit eye. *J. Pharm. Sci.* **68**:435–443 (1979).

3. V. H. L. Lee and J. R. Robinson. Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits. *J. Pharm. Sci.* **68:**673–684 (1979).
4. S. S. Chrai and J. R. Robinson. Corneal permeation of topical pilocarpine nitrate in the rabbit. *Am. J. Ophthalmol.* **77:**735–739 (1974).
5. P. H. Chung, T. F. Chin, and J. L. Lach. Kinetics of the hydrolysis of pilocarpine in aqueous solution. *J. Pharm. Sci.* **59:**1300–1306 (1970).
6. S. C. Miller and Thomas F. Patton. Age-related differences in ophthalmic drug disposition I. Effect of size on the intraocular tissue distribution of pilocarpine in albino rabbits. *Biopharm. Drug. Dispos.* **2:**215–233 (1981).
7. N. Keller, D. Moore, D. Carper, and A. Longwell. Increased corneal permeability induced by the dual effects of transient tear film acidification and exposure to benzalkonium chloride. *Exp. Eye Res.* **30:**203–210 (1980).
8. S. C. Miller. Ocular pharmacokinetics of pilocarpine in albino rabbits I. Age related differences II. Physiological modeling of intraocular disposition. Ph.D. thesis, University of Kansas, 1980.
9. T. F. Patton. Pediatric dosing considerations in ophthalmology: dosage adjustments based on aqueous humor volume ratio, *J. Pediatr. Ophthalmol.* **14:** 254–256 (1977).
10. R. G. D. Steel and J. W. Torrie. *Principles and Procedures of Statistics,* McGraw-Hill, New York, 1960.
11. S. S. Chrai, T. F. Patton, A. Mehta, and J. R. Robinson. Lacrimal and instilled fluid dynamics in rabbit eyes, *J. Pharm. Sci.*, **62:** 1112–1121 (1973).
12. S. C. Miller, R. D. Gokhale, T. F. Patton, and K. J. Himmelstein. Pilocarpine ocular distribution volume. *J. Pharm. Sci.* **69:**615–616 (1980).
13. J. H. Prince (ed.), *The Rabbit in Eye Research,* Charles C. Thomas, Springfield, Ill., 1964.
14. S. S. Hayreh. Posterior drainage of the intraocular fluid from the vitreous. *Exp. Eye Res.* **5:**123–144 (1966).
15. A. Bill. Quantitative Determination of Uveal Blood-Flow in Rabbits. *Acta Physiol. Scand.* **55:**101–110 (1962).
16. T. F. Patton. Pharmacokinetic evidence for improved ophthalmic drug delivery by reduction of instilled volume. *J. Pharm. Sci.* **66:**1058–1059 (1977).
17. B. Carnahan, H. A. Luther, and J. O. Wilkes. *Applied Numerical Methods,* John Wiley & Sons, New York, 1969.
18. E. L. Parrott. *Pharmaceutical Technology, Fundamental Pharmaceutics,* Burgess Publishing Co., Minneapolis, 1970.